Characterization of Sericin Powder Prepared from Citric Acid-degraded Sericin Polypeptides of the Silkworm, *Bombyx Mori*

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Received July 24, 2003; Accepted January 20, 2004

Acid-degraded sericin powder (AC-SP) was prepared from aqueous solution containing citric acid-degraded sericin polypeptides of *Bombyx mori*. The morphological and biochemical properties of AC-SP were compared with those of alkali-degraded sericin powder (AL-SP) and hot-water degraded sericin powder (HW-SP). Based on an SEM analysis, AC-SP showed a thin film structure of 10–100 μm with good dispersity while AL-SP and HW-SP had a much larger thin film structure (<500 μm). The extract of AC-SP showed stronger trypsin inhibitor activity due to cocoon shell trypsin inhibitor (CSTI-IV) than that of HW-SP. The extract of AL-SP showed no CSTI-IV activity. It was found that AC-SP was a trypsin inhibitor complex powder and that the release of CSTI-IV from AC-SP depended on pH and ion strength. Similar powder materials were obtained when such organic acids as tartaric acid and succinic acid were used. These results suggest that the acid-degraded sericin polypeptides work as a protein matrix to which CSTI-IV may bind ionically.

Key words: sericin polypeptides; *Bombyx mori* cocoon; sericin powder; citric acid; trypsin inhibitor

Sericin, the second major *B. mori* silk protein, has excellent moisture-absorbing and -desorbing properties that render it suitable for use as an additive in cosmetics. This feature is mainly attributed to the high content of serine (30 mol%). Sericin has been shown to act as an antioxidant protein that inhibits tyrosinase activity and antioxidative stress was recently found to be essential in suppressing tumor promotion in mouse colon and skin. It has been shown to act as an antioxidant protein that inhibits tyrosinase activity and lipid peroxidation in vitro, and its ability to reduce oxidative stress was recently found to be essential in suppressing tumor promotion in mouse colon and skin. The high content of the hydroxyl groups of sericin was recently shown to provide anti-freezing activity through a close examination of a purified recombinant serine-rich peptide (Ser 45%) occurring repetitively in a sericin protein that is encoded in the *Ser1* gene. But study of the structural-functional relationship of sericin is limited due to its heterogeneity and impurities caused by the degumming procedure. Sericin is hydrolytically degummed from fibroin under alkaline conditions. Waste sericin containing sericin hydrolysates, or degraded polypeptides, is a by-product discarded approximately in the amount of 50,000 tons worldwide each year during the process of degumming raw silk. To date, many attempts have been made to recycle waste sericin produced by the silk degumming process. In some cases, methods have been developed and patented for recovering degraded sericin polypeptides from wastewater at a high yield not only to reduce the environmental impact but also to utilize them as a biomaterial with industrial potential. The degumming of silk has been carried out for many years in alkaline solutions on a commercial scale, although the effectiveness of various organic acids has been reviewed in some detail. Therefore more attention has been focused on the practical use of alkaline-degraded sericin polypeptides and very little is known about the properties of acid-degraded sericin polypeptides.

In addition to being a raw material for silk, the *B. mori* cocoon has recently attracted attention as a bioresource because it contains non-fibrous components, such as flavonoids, uric acid, and protease inhibitors. It has been suggested that these components might be a defense agent that protects silk from oxidative stress or microbial degradation. The aqueous cocoon extract abundant in the non-fibrous components is therefore considered a crude mixture with biological significance. Some Kunitz-type protease inhibitors are known to function as biomodulators that control various processing enzymes related to tissue repair and tumor progression, such as hepatocyte growth factor activator and matriptase. The crude cocoon extract is thus worth recovering because some of the cocoon shell protease inhibitors (CSTIs) belong to the Kunitz-type family. Most of the CSTIs in the cocoon are present in the layer of sericin. However, in recovering CSTI, alkali degumming may be harsher than acid degumming. Thus, the utilization of CSTI, along with degraded sericin polypeptides, has not been satisfactory.

Abbreviations: CSTI, cocoon shell trypsin inhibitor; DPPH, 2,2′-diphenyl-1-picyrylhydrazyl; AC-SP, acid-degraded sericin powder; AL-SP, alkali-degraded sericin powder; HW-SP, hot-water degraded sericin powder; Et-SP, ethanol-precipitated sericin powder; BAPNA, 4-n-benzoyl-L-arginine-p-nitroanilide; BPTI, bovine pancreatic trypsin inhibitor

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2 N,N′-diphenyl-

3 N,N′-diphenyl-

4 N,N′-diphenyl-

5 N,N′-diphenyl-

6 N,N′-diphenyl-

7 N,N′-diphenyl-

8 N,N′-diphenyl-

9 N,N′-diphenyl-

10 N,N′-diphenyl-

11 N,N′-diphenyl-

12 N,N′-diphenyl-

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14 N,N′-diphenyl-

15 N,N′-diphenyl-

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In this report, acid-degraded sericin polypeptide powder (AC-SP) was prepared under acidic conditions using citric acid, and its morphological and biochemical properties were compared with those of alkali-degraded sericin powder (AL-SP) and hot-water degraded sericin powder (HW-SP).

Materials and Methods

Preparation of sericin powders. Freshly spun cocoons were obtained from the commercial race Kinsyu × Showa. The larvae were reared at 25–27 °C on mulberry leaves. Aqueous sericin solution was prepared by acid degumming using citric acid according to the method described by Shiozaki et al. (2000). Seventeen to 18 clean cocoons (9 g) were chopped into small pieces and boiled for 30 min in 160 ml of 1.25% citric acid solution. Tartaric acid and succinic acid were also used in the same manner to prepare aqueous sericin solutions. After removing insoluble fibers by paper filtration, the clear filtrate was immediately dialyzed against distilled water for three days using either 2000-, 15,000-, or 50,000-molecular cutoff cellulose tubing (Spectra/Por Dialysis Membrane, Spectrum®). Proteins aggregated in the nondialyzable fractions (2 K, 15 K, and 50 K) were disaggregated by ultrasonification. Each slurry was lyophilized to dryness, and the lyophilizate powders (AC-SP-2 K, -15 K, and -50 K) were stored in a desiccator at -20 °C overnight and then lyophilized to dryness. The lyophilizate powders (AC-SP-2 K unless otherwise specified) were lyophilized to dryness, and the lyophilizate powders (AC-SP-2 K unless otherwise specified) were lyophilized to dryness. The lyophilizate powders (AC-SP-2 K unless otherwise specified) were lyophilized to dryness. The lyophilizate powders (AC-SP-2 K unless otherwise specified) were lyophilized to dryness. The lyophilizate powders (AC-SP-2 K unless otherwise specified) were lyophilized to dryness. The lyophilizate powders (AC-SP-2 K unless otherwise specified) were lyophilized to dryness. The lyophilizate powders (AC-SP-2 K unless otherwise specified) were lyophilized to dryness. The lyophilizate powders (AC-SP-2 K unless otherwise specified) were lyophilized to dryness. The lyophilizate powders (AC-SP-2 K unless otherwise specified) were lyophilized to dryness.

HW-SP was produced from the aqueous sericin solution prepared by alkali degumming using 0.5% sodium carbonate. For HW-SP, aqueous sericin solutions were extracted with hot water under pressure at 110, 115, and 121 °C for 30 min using an autoclave to prepare HW-SP-110, -115, and -121 respectively.

SEM analysis. A morphological study was carried out using a JEOL scanning electron microscope (JSM-6100). Samples were sprinkled on the double-sided tape attached to a specimen stub and coated with a thin gold layer using a JEOL ion sputter (JFC-1100E).

Measurement of powder color. Powder color was measured using a Minolta CM 508i reflectance spectrophotometer in terms of the Commission International d’Eclairage (CIE) Lab system, where any color can be represented by three color variables: L*, a*, and b*. L* is a measure of brightness, and L* 100 indicates perfect reflectivity, or white; a* and b* demonstrate red-green and yellow-blue chromacity respectively. Positive a* and b* values represent increased redness while positive b* values indicate increased yellowness. Powder samples (1 g) were packed in a transparent polyethylene bag (7 x 11 cm) and the intensity of reflected light was measured. Measuring conditions for outdoor daylight were an observation angle of 10 with D65 illuminant (D65/10°). The D65 illuminant corresponds to natural daylight with a correlated color temperature of 6504 degrees Kelvin (K). For indoor illumination, observation was carried out by angle 2 with C illuminant corresponding to indirect sunlight of 6774 K (C/2°). The daylight whiteness of powders was described by the CIE-whiteness index (WI). Indoor whiteness and yellowness indexes were automatically calculated using the CIE XYZ color standard and expressed by the ATSM (American standard for testing and materials) E313-WI and ASTM E313-YI respectively.

HPLC analysis. Peptides were extracted four times from AC-SP (50 mg) with 1 ml of distilled water at 25 °C, followed by subsequent extraction at 60 °C for 30 min.Each extract was lyophilized and then dissolved in 200 µl of 10% acetonitrile containing 0.1% trifluoroacetic acid (eluent A) for HPLC analysis. Aliquots (50 µl) were analyzed by reversed-phase HPLC using a WakoSil 5C8 column (Wako Pure Chem.). The elution was carried out by a linear gradient of eluent A from 10 to 30% in 50 min, at a flow rate of 1 ml/min. Peptides were monitored at 215 nm.

Measurement of trypsin inhibitor activity. Tryptsin inhibition was measured with the p-nitroanilide assay as described previously. Samples of powder (10 mg) were dispersed in 1 ml of 100 mM Tris-HCl buffer solution (TBS) (pH 8.3), and clear supernatant was obtained by centrifugation (5,000 × g) for 10 min at 10 °C. Aliquots (10–150 µl) of the supernatant were added to 1 ml of 100 mM TBS (pH 8.3) containing 2 mM CaCl2, 5 µg bovine trypsin, and 1 mM N-alpha-benzoyl-DL-arginine-p-nitroanilide (BAPNA). After incubating the mixture at 37 °C for 30 min, the reaction was terminated by the addition of 0.5 ml of 30% (V/V) acetic acid, and the liberated p-nitroaniline was measured spectrophotometrically at 410 nm. One unit of inhibitor was defined as the volume of supernatant that caused a 50% inhibition of BAPNA hydrolysis by 5 µg of bovine trypsin.

Results

Morphological features of sericin powders

The yield of sericin powder obtained by acid degumming was comparable to that by alkali and hot-water degumming. The average cocoons weight loss for citric acid degumming was 27.8% (n = 3, s.d. 2.29), while those for alkali degumming using 0.5% sodium carbo-
nate solution and hot-water (110°C) degumming were 25.7% (n = 5, s.d. 0.23) and 21.0% respectively. Figure 1 shows SEM images of AC-SP in comparison with those of AL-SP. Small, thin films with a leaf-like structure rather than fine particles were observed in AC-SP (Fig. 1-a). These leaf-like films were approximately 50–100 μm in size. Rod-like microstructures were also observed along with the leaf-like films in the image. Similar morphological features were observed in the powders prepared by tartaric and succinic acid degumming (images not shown). Based on a higher magnification image (×3,000) (Fig. 1-b), the diameter of the rod-like structures was approximately 2 μm. In Fig. 1-b, spherical structures less than 3 μm in diameter were also observed. These microspheres may be closely associated with the rod-like structures since most of the microspheres adhered to the surface of the rod-like structures. The microspheres and rod-like structures of AC-SP appeared to be relatively soluble since no such microstructures were observed after immersion in distilled water (Fig. 1-c). Figure 1-d shows an image of AL-SP. The image exhibited the presence of a thin-film structure, the size of which, however, was much larger (<500 μm) than that of AC-SP. Moreover, there was no indication of the microstructures identified in AC-SP. SEM images of HW-SP-110, -115, and -121 showed a similar feature (image not shown) to AL-SP.

In contrast to AC-SP, Et-SP showed porous solid particles approximately 10–30 μm in diameter (Fig. 2-a). This porous structure was shown to consist of much smaller particles measuring approximately 1 μm in diameter based on a finer level of SEM analysis (×8,000) (Fig. 2-b).

Comparison of powder color between AC-SP and AL-SP

AC-SP was lighter and whiter in color than AL-SP. To clarify the color difference, colorimetry of AC-SP and AL-SP in terms of the CIE L*a*b* color system was carried out. The tristimulus values along with the whiteness and yellowness indexes are summarized in Table 1. The L* indexes of AC-SP for outdoor daylight (D65/10°) and indoor illumination (C/2°) were 90.93 and 91.05 respectively, indicating that the color of AC-SP was nearly white. AL-SP was slightly dull and yellowish in color, and the L* indexes for D65/10° and C/2° were 89.28 and 89.47 respectively. AC-SP had whiteness index values of 59.0 for CIE-WI and 58.19 for

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<th>AC-SP (n = 3)</th>
<th>AL-SP (n = 3)</th>
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<tr>
<td>D65/10°</td>
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<tr>
<td>Brightness, L*</td>
<td>90.93 0.15</td>
<td>89.28 0.37</td>
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<td>Redness, a*</td>
<td>−0.16 0.06</td>
<td>0.14 0.09</td>
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<tr>
<td>Yellowness, b*</td>
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<td>4.16 0.32</td>
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<tr>
<td>CIE-WI</td>
<td>59.00 1.26</td>
<td>43.98 1.34</td>
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<td>ASTME313-WI</td>
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Fig. 1. SEM Images of Freeze-dried Sericin Powder.
(a)–(c) AC-SP; (d) AL-SP. Scale bars in (a) and (d) represent 100 μm, and those in (b) and (c), 10 μm.

Fig. 2. SEM Image of Et-SP.
Scale bars in (a) and (b) indicate 10 μm and 1 μm respectively.
ASTME313-WI C/2, while AL-SP was less white with CIE-WI values of 43.98 for D65/10 and 44.26 for ASTM E313-WI C/2. The b* indexes of AL-SP were 6.45 (D65/10) and 6.54 (C/2). The yellowness ASTM E313-YI value of AL-SP was 10.25, 58.4% greater than that of AC-SP. Taken together, these results indicate a relative presence of the yellow pigment that contributed to the creamy-yellow color of AL-SP. The cocoon used in this study was white in color and had a trace amount of pigments such as flavonoids and carotenoids. Therefore the relatively high yellowness value of AL-SP may be due to colored compounds generated during degumming under alkali conditions.

Analysis of soluble polypeptides of AC-SP
AC-SP exhibited a milky white dispersion stable at least 15–20 min in water while AL-SP and HW-SP precipitated immediately after being immersed. AC-SP had a water-solubility value of 22% at 60°C, suggesting the presence of water-soluble polypeptides. Figure 3 shows HPLC profiles for the water-soluble fractions that were obtained by sequentially extracting four times from AC-SP at 37°C. In the first extract (Fig. 3-a), the presence of some water-soluble polypeptides from AC-SP is shown. Most of the polypeptide peak fractions, including I, II, and III, were detected during the first 20 min on a C8 column. The elution of these polypeptides from AC-SP gradually decreased following a series of extractions (Fig. 3-b and c), and the polypeptides of the final extraction were almost undetectable (Fig. 3-d) using the same column. After subsequent extraction with hot water (60°C), a similar HPLC profile to Fig. 3-a was obtained as shown in Fig. 3-e. But the proportion of the HPLC profile was distinct from that shown in Fig. 3-a in fractions I and III. The polypeptide peaks in fraction I were undetectable, and a marked increase was seen in the intensity of the peaks in fraction III. In contrast, the intensities of fraction II under both extraction conditions were comparable. These results indicate that AC-SP has some water-soluble polypeptides of different solubility.

Trypsin inhibitory activity in sericin powders
Figure 4 shows the trypsin inhibitor activity in AC-SP, AL-SP, and HW-SP-110. Aqueous extract from AC-SP and HW-SP-110 demonstrated trypsin inhibitor activity due mostly to CSTI-IV, one of the previously reported iso-inhibitors of the cocoon. CSTI-IV was detected in at most five min when dispersed into water, and the total activity recovered from AC-SP was not affected by extracting temperatures. CSTI-IV activity in HW-SP-110 was 31%, which was 60% lower than that in AC-SP. In the case of AL-SP, no CSTI-IV activity was detected. In order to quantitatively evaluate trypsin inhibitor activity in AC-SP, CSTIs were directly extracted from the cocoon with 100 mM TBS (pH 8.3) and assayed as a standard for bovine trypsin inhibition.

![Fig. 3. C8-HPLC Profiles of Water-soluble Polypeptides of AC-SP.](image)

Water-soluble polypeptides were sequentially extracted four times from 50 mg of AC-SP with distilled water at 37°C. After the sequential extractions, the sample was rinsed at 60°C. Each extract was concentrated and analyzed on a Wakosil SC8 column. (a) first extraction; (b) second extraction; (c) third extraction; (d) fourth extraction; (e) hot-water extraction at 60°C.

![Fig. 4. Trypsin Inhibitor Activity in AC-SP, AL-SP, and HW-SP.](image)

Ten mg of the sericin powders was immersed in 1 ml of 100 mM Tirs-HCl buffer (pH 8.3) for 5–50 min at 37°C and centrifuged. Aliquots (50 μl) of each supernatant were assayed for trypsin inhibition. In some experiments, AC-SP was treated at 60°C for 70 min. The data shown are means ± SD from three independent experiments.
The total trypsin inhibitor activity recovered from an average 5.58 g cocoon was estimated to be 4592 units. The specific activity unit for the standard was thus calculated to be 823 unit/g cocoon. Trypsin inhibition curves for the AC-SPs and Et-SP are presented in Fig. 5. CSTI-IV was recovered efficiently from aqueous sericin solution by 2 kDa-molecular-cutoff dialysis. The specific unit of trypsin inhibitor activity in AC-SP-2 K was calculated to be 813.9 unit/g cocoon (2,940 unit/g powder). Powders from AC-SP-15 K and AC-SP-50 K showed less CSTI-IV activity, with 2,450 and 2,128 unit/g powder respectively. These values were unexpectedly high considering the molecular size of CSTI-IV (6 kDa). Et-SP showed the lowest inhibition (1,000 unit/g powder). The volume of supernatant of Et-SP required for complete inhibition of 5 g bovine trypsin was about 150 l, more than twice as high as that of AC-SP-2 K. The activity of CSTI-IV in AC-SP was recognized shortly after immersion into 100 mM TBS (pH 8.3), suggesting that CSTI-IV was quite rapidly solubilized or released in the buffer. When dialyzed using 50 kDa-molecular-cutoff tubing, most of the CSTI-IV in aqueous sericin solution was retained by the tubing. It was speculated that there may be a protein-protein interaction to form a complex between CSTI-IV and the sericin polypeptides.

Figure 6 shows trypsin inhibition curves for HW-SP-110, -115, and -121. The inhibitor activity of HW-SP-115 and -121 decreased approximately 60% and 75% respectively compared to that of HW-SP-110. This inactivation may be attributed to heat treatment.

**Release of CSTI-IV from AC-SP and HW-SP-110**

In order to elucidate the mechanism of the release of CSTI-IV, AC-SP and HW-SP-110 were subjected to buffers of various concentrations (1–100 mM) and pH ranges of 5.5–9.0. Figure 7 shows the effect of TBS concentration on the release of CSTI-IV in terms of trypsin inhibition. The release of CSTI-IV from AC-SP increased with an increasing concentration of TBS. Trypsin inhibition activity was seen to reach a maximum in 100 mM TBS, which was nearly four times greater than that in 1.0 mM TBS. Although trypsin inhibition for HW-SP-110 was comparable to that for AC-SP in distilled water, there was no remarkable increase in trypsin inhibitor activity with increasing ion strength. The release of CSTI-IV from AC-SP was not influenced by the presence of nonionic detergent such as 0.1% NP-40. The percentage inhibition of trypsin (5 μg) by 10 mg AC-SP in 0.1% NP-40 was 20.1%, similar to that in distilled water. This suggests that there was no hydrophobic interaction between CSTI-IV and AC-SP. Figure 8 demonstrates the effect of pH on the release of CSTI-IV. At higher pH values (>7.2), potent trypsin inhibition
Fig. 8. Effect of pH on the Release of CSTI-IV from AC-SP and HW-SP-110.

Samples (10 mg) of the sericin powders were dispersed in 100 mM ammonium acetate buffer (pH 3.9 and 3.5) and 100 mM Tris-HCl buffer (pH 7.2, 8.3, and 9.0). After centrifugation, the supernatant (50 μl) was assayed for trypsin inhibition. Dark bars, AC-SP; open bars, HW-SP-110. The data shown are means ± SD from three independent experiments.

Discussion

In the present study, we prepared various sericin powders from degraded sericin polypeptide and examined their morphological and biochemical properties. Citric acid was found to be an effective reagent in extracting CSTI-IV with high recovery (~98%), together with degraded sericin polypeptides from the cocoon. Furthermore, AC-SP was demonstrated for the first time to be a complex material that can hold CSTI-IV mainly via ionic bonding. CSTI-IV, which is homologous to bovine trypsin inhibitor (BPTI), is a heat and acid-stable protein.8) But it was found that HW-SP-115 and -121 showed relatively weak trypsin inhibitor activity. In AL-SP, CSTI-IV was completely inactivated. Thus degumming with alkali and hot water at higher temperatures (~115 °C) was not suitable for preparing CSTI complex powder. Citric acid-treated sericin was found to be a mixture of at least 15 degraded polypeptides differing in pl based on iso-electrofocusing (data not shown). Some of the acid-degraded sericin polypeptides of AC-SP were water soluble, and their solubility to water was dependent on temperature. It remains unknown, however, whether the differences in solubility correlate with factors such as molecular size distribution, macromolecular structure, or polypeptide hydrophobicity.

AC-SP has some unique properties that make it widely distinct from AL-SP in terms of color and powder size, as well as dispersity. AC-SP was seen to be a bright and colorless powder compared to AL-SP. It was demonstrated that hydrolyzation of sericin in a hot alkal solution for an extended period of time (60–240 min) caused the generation of remarkable yellowish browning and fluorescence (unpublished data). These phenomena during alkali treatment may be due to a melanoidin-like compound resulting from the Maillard, or sugar-amine reaction, since sericin is a glycoprotein that has two types of oligosaccharide units.20) Alkali-degraded sericin also exhibited DPPH free radical scavenging activity. This scavenging activity increased with increasing extraction time (unpublished data). These results confirm that the brown pigment in AL-SP may be a Maillard reaction product, which is known to function efficiently to quench various free radicals.21) In acid degumming, on the other hand, the Maillard reaction was not likely to be proceeded in AC-SP, where no DPPH radical quenching occurred (data not shown).

In addition to its high molecular weight (~400 kDa), sericin is well characterized by a gelatinous protein due to the high content of the hydroxyl groups in serine and carbohydrate units. In acid-degraded sericin polypeptides, gelatin-like materials were spontaneously formed in 4–6 hours at room temperature. These gels were stable for at least 5–8 months at 10 °C and could be readily resolubilized by heating at 98 °C in the presence of 1.25% citric acid. In contrast, sericin polypeptides treated with alkali or hot water (110 °C) showed neither a reversible gel-sol transformation nor the fine leaf-like structure identified in AC-SP. The relationship between the powder morphology and phase transformation is presently unknown. The leaf-like structure identified here was observed exclusively when sericin was treated with the organic acids. It has been suggested that the mechanism of acid hydrolysis of proteins involves specific cleavage at the aspartic and glutamic acid residues.8) Sericin contains about 16.7 mol% aspartic acid and 4.7 mol% glutamic acid. Therefore the formation of the acid-specific microstructure may be closely related to the limited proteolytic cleavage by the organic acid treatment.

We demonstrated, for the first time, that AC-SP may function as a protein matrix having anion exchanging capability. CSTI-IV, an acidic protein with a pl value 4.3,12) is negatively charged in neutral or alkaline solutions. Therefore we speculated that the CSTI-IV in AC-SP may be incorporated into the AC-SP matrix through ionic bonding, since the release of CSTI-IV from AC-SP depends profoundly on the pH or concentration of a buffer solution. The acid-degraded sericin polypeptides covered a broad pl range from 3.5 to 6.0.
Therefore, at higher pH values (>7.2), most of the polypeptides of AC-SP, which existed as a negatively charged matrix, are likely to release CSTI-IV. We have also shown that there were some CSTI-IV that may weakly interact with AC-SP. At lower pH (<5.5) in a 100 mM ammonium acetate buffer, nearly 50% of the CSTI-IV was released from the AC-SP matrix. Similarly, approximately 20% was liberated when AC-SP was immersed into distilled water or a detergent. The presence of CSTI-IV differing in adsorbing properties might be attributed to the heterogeneity of matrix polypeptide. Further purification of the acid-degraded sericin polypeptides is necessary in order to elucidate the physicochemical interaction of the sericin polypeptides with CSTI-IV. Further identification and elucidation of a specific binding site(s) for CSTI-IV, which is rich repetitive sequence in silk protein sericin. J. Dainippon Silk Foundation colour, 20, 1007–1015 (2002).

References


